


IN THE CLAIMS:

The claims are reiterated for the convenience of the Examiner.

Please amend the claims as follows:

1. A method for high throughput screening of prokaryotic genomic DNA samples to identify one or more enzymes encoded by the prokaryotic DNA of said sample, comprising the steps of ;
 - a) generating a normalized, multispecific, prokaryotic expression library;
 - b) inserting bioactive substrates into samples of the library;
 - c) screening the samples with a fluorescent analyzer that detects bioactive fluorescence;
 - d) separating samples detected as positive for bioactive fluorescence; and
 - e) determining the DNA sequence of positive samples;wherein the DNA sequence identifies and encodes an enzyme that catalyzes the bioactive substrate detected in step d).
2. The method of claim 1, wherein the enzyme is selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.
3. The method of claim 1, wherein the prokaryotic expression library contains at least of about 2×10^6 clones.
4. The method of claim 1, wherein the sample is a prokaryotic cell.
5. The method of claim 4, wherein the prokaryotic cell is gram negative.
6. The method of claim 1, wherein the sample is encapsulated in a gel microdrop.

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7. The method of claim 1, wherein the high-throughput screening step c) screens up to about 35 million samples per hour.
 8. The method of claim 1, wherein the prokaryotic expression library contains extremophiles.
 9. The method of claim 3, wherein the extremophiles are thermophiles.
 10. The method of claim 3, wherein the extremeophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.
 11. The method of claim 1, wherein the bioactive substrate comprises C12FDG.
 12. The method of claim 10, wherein the bioactive substrate further comprises a lipophilic tail.
 13. The method of claim 1, wherein the samples are heated before step b).
 14. The method of claim 13, wherein the heating is in the range of about 70°C.
 15. The method of claim 14, wherein the heating occurs in the range of about 30 minutes.
 16. The method of claim 1, wherein the fluorescent analyzer comprises a FACS apparatus.
 17. The method of claim 1, wherein the prokaryotic expression library is biopanned before step b).

18. (Amended) The method of claim 1, including [the additional steps of :] subjecting a[n] first enzyme encoded by the DNA identified in step d), in order to [directed evolution] obtain an enzyme with an altered activity, comprising [the steps of]:

- a) [subjecting] introducing at least one mutation into the DNA encoding the first enzyme [to] by non-directed mutagenesis; and
- b) comparing the enzyme activity of a DNA expression product from a) with the activity of the first enzyme, wherein a difference in activity is indicative of an effect of at least one mutation, thereby providing an enzyme with an altered activity [screening mutant enzymes produced in step a) for a mutant enzyme that is stable at a temperature of at least in the range of about 60°C and that has functioning enzymatic activity at a temperature at least 10°C below its optimal temperature range and that catalyzes a greater amount of a catalytic substrate per a defined unit of time than the enzyme of step a)].

As amended, claim 18 reads as follows:

18. The method of claim 1, including subjecting a first enzyme encoded by the DNA identified in step d), in order to obtain an enzyme with an altered activity, comprising:

- a) introducing at least one mutation into the DNA encoding the first enzyme by non-directed mutagenesis; and
- b) comparing the enzyme activity of a DNA expression product from a) with the activity of the first enzyme, wherein a difference in activity is indicative of an effect of at least one mutation, thereby providing an enzyme with an altered activity.